CLAIMS

1. Use of a fluorescent protein chosen from fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, this protein being chosen in particular from:

– green fluorescent protein (GFP), or

– variants derived from GFP by addition, deletion or substitution of one or more amino acids, with the proviso that these variants conserve the fluorescence property,

– or fragments of GFP, or fragments of the above-mentioned variants, with the proviso that these fragments conserve the fluorescence property,

for the detection and quantification of non-covalent interactions between a target protein labeled with GFP or one of the variants defined above or one of the fragments defined above and one of its ligands labeled with a label consisting:

– either of a molecule which is capable of absorbing the light emitted by the fluorescent protein,

– or of a fluorescent substance,

this detection and quantification taking place by fluorescence energy transfer:

• between GFP or one of the variants defined above, or one of the fragments defined above, and the above-mentioned fluorescent substance, the fluorescent substance being such that either it is excitable at the emission wavelength of GFP or of one of the above-mentioned variants, or of one of the above-mentioned fragments, or it emits at the excitation wavelength of GFP, or of one of the above-mentioned variants, or of one of the above-mentioned fragments, or

• in between GFP or one of its variants defined above, or one of the fragments defined above, and the above-mentioned molecule which is capable of absorbing the light emitted by the fluorescent protein.

2. Use of a ligand labeled with a label consisting:

– either of a molecule which is capable of absorbing the light emitted by the fluorescent protein,

– or of a fluorescent substance,

for the detection and quantification of non-covalent interactions between a target protein and the above-mentioned ligand, the said target protein being labeled with a fluorescent protein chosen from fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, the molar extinction coefficient of which is greater than about 14,000 $M^{-1}cm^{-1}$ and the quantic fluorescence yield is greater than about 0.38, this protein being chosen in particular from:

- green fluorescent protein (GFP), or
- variants derived from GFP by addition, deletion or substitution of one or more amino acids, with the proviso that these variants conserve the fluorescence property,
- or fragments of GFP, or fragments of the above-mentioned variants, with the proviso that these fragments conserve the fluorescence property,

this detection and quantification taking place by fluorescence energy transfer:

- between GFP or one of the variants defined above, or one of the fragments defined above, and the above-mentioned fluorescent substance, the fluorescent substance being such that either it is excitable at the emission wavelength of GFP or of one of the above-mentioned variants, or of one of the above-mentioned fragments, or it emits at the excitation wavelength of GFP, or of one of the above-mentioned variants, or of one of the above-mentioned fragments, or
- between GFP or one of its variants defined above, or one of the fragments defined above, and the above-mentioned molecule capable of absorbing the light emitted by the fluorescent protein.

3. Use according to claim 1, wherein the fluorescent protein is chosen from:

- green fluorescent protein (GFP),
- cyan fluorescent protein (CFP),
- yellow fluorescent protein (YFP),
- GFPUV,

or mutants thereof in which the codons are optimized for expression in human, bacterial or plant cells.

4. Use of a fluorescent protein (No 1) according to either of claims 1 and 3, wherein the ligand is labeled

* either with a fluorescent substance, the labeling being carried out:

– either via a chemical route, the fluorescent substance then being a chemical compound,

– or via a recombinant route, the fluorescent substance then being a fluorescent peptide or protein (No 2) which can be chosen in particular from the fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, this fluorescent substance being chosen in particular from:

– green fluorescent protein (GFP), or

– variants derived from GFP by addition, deletion or substitution of one or more amino acids, with the proviso that these variants conserve the fluorescence property,

– or fragments of GFP, or fragments of the above-mentioned variants, with the proviso that these fragments conserve the fluorescence property,

* or with a non-fluorescent substance belonging to the Acid Violet group [Acid Violet 5, CAS 10130-48-0 ; Acid Violet 7, CAS 4321-69-1 ; Acid Violet 17, CAS 4129-84-4], the Acid Red group [Acid Red 1, CAS 3734-67-6 ; Acid Red 8, CAS 4787-93-3 ; Acid Red 37, CAS 6360-07-2 ; Acid Red 40, CAS 12167-45-2 ; Acid Red 106, CAS 6844-74-2 ; Acid Red 114, CAS 6459-94-5], alizarins, aluminon, azocarmine B [CAS 25360-72-9], basic fuschin [Basic Red 9, CAS 569-61-9], Bordeaux R [Acid Red 17, CAS 5858-33-3] and Carmine [CAS 1390-65-4].

5. Use of a fluorescent protein according to one of claims 1, 3 or 4, wherein the target protein is labeled with the YP or EGFP protein, and the ligand is labeled with the BFP protein, or the target protein is labeled witg the BFP protein, and the ligand is labeled with the YP or EGFP protein.

6. Use, according to claim 1, of a fluorescent protein chosen from the fluorescent proteins obtained or derived from the autofluorescent proteins of cnidarians, this protein being chosen in particular from:

– green fluorescent protein (GFP), or

– variants derived from GFP by addition, deletion or substitution of one or more amino acids, with the proviso that these variants conserve the fluorescence property,

– or fragments of GFP, or fragments of the above-mentioned variants, with the proviso that these fragments conserve the fluorescence property,

for the detection and quantification of non-covalent interactions between a target protein labeled with GFP or one of the variants defined above or one of the fragments defined above and one of its ligands labeled with a fluorescent substance, this detection and quantification taking place by fluorescence energy transfer between GFP or one of the variants defined above, or one of the fragments defined above, and the said fluorescent substance, the fluorescent substance being such that either it is excitable at the emission wavelength of GFP or of one of the above-mentioned variants, or of one of the above-mentioned fragments, or it emits at the excitation wavelength of GFP, or of one of the above-mentioned variants, or of one of the above-mentioned fragments.

7. Use according to one of claims 1 to 6, wherein the fluorescent protein is EGFP and wherein:

– either the EGFP is a fluorescence energy donor and the label absorbing the light emitted by the EGFP is a fluorescent or non-fluorescent substance, and the marker being chosen from substances whose excitation spectrum overlaps the emission spectrum of EGFP, and in particular, when the label is a fluorescent substance, it is chosen from: 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (Bodipy), eosin, erythrosin, tetramethylrhodamine, sulphorhodamine 101 sold by Molecular Probe under the name Texas Red, and derivatives thereof which, on the one hand, allow grafting, and, on the other hand, have an excitation spectrum which overlaps the emission spectrum of EGFP,

and, when the label is not a fluorescent substance, it is chosen from the Acid Violet group [Acid Violet 5, CAS 10130-48-0 ; Acid Violet 7, CAS 4321-69-1 ; Acid Violet 17, CAS 4129-84-4], the Acid Red group [Acid Red 1, CAS 3734-67-6 ; Acid Red 8, CAS 4787-93-3 ; Acid Red 37, CAS 6360-07-2 ; Acid Red 40, CAS 12167-45-2 ; Acid Red 106, CAS 6844-74-2 ; Acid Red 114, CAS 6459-94-5], alizarins, aluminon, azocarmine B [CAS 25360-72-9], basic fuschin [Basic Red 9, CAS 569-61-9], Bordeaux R [Acid Red 17, CAS 5858-33-3] and Carmine [CAS 1390-65-4],

– or the EGFP is a fluorescence energy acceptor and the fluorescent substance is a fluorescence energy donor and is chosen from substances whose emission spectrum overlaps the excitation spectrum of EGFP, and in particular from: coumarins, fluorescamine, 6-(N-methylanilino)naphthalene , (mansyl) and derivatives thereof which, on the one hand, allow grafting, and, on the other hand, have an excitation spectrum which overlaps the emission spectrum of EGFP,

– or the fluorescent protein is BFP and is a fluorescence energy donor and the fluorescent substance is an energy acceptor and is chosen from fluorescein and 7-nitro-2-benzoxa-1,3-diazole,

– or the fluorescent protein is BFP and is a fluorescence energy acceptor and the fluorescent substance is an energy donor and is chosen from pyrene and coumarin or derivatives thereof which, on the one hand, allow grafting, and, on the other hand, have an excitation spectrum which overlaps the emission spectrum of ECFP.

8. Use according to one of claims 1 to 7, in which the target protein is chosen from:

– membrane-bound receptors coupled to protein G in particular in Supplement Trends in Pharmacological Sciences, 1997 (*Receptor and ion Channel Nomenclature*),

– growth factor receptors, in particular those which are structurally linked to the insulin receptor (Yarden, Y. and Ullrich, A. 1988, Biochemistry 27:3113-3119) or to the γ interferon receptor (Brisco, J. *et al.* 1996, Phylos. Trans. R. Soc. Lond. B. Biol. Sci. 351:167-171 ; Ihle, J.N. 1995, Nature 377:591-594),

– ion channel receptors, in particular in Supplement Trends in Pharmacological Sciences, 1997 (*Receptor and ion Channel Nomenclature*),

– intracellular nuclear receptors, in particular those which are structurally linked to the steroid receptor (Mangelsdorf et *al.* 1995, Cell, 83:835-839 ; Wurtz, J.L. et *al.* 1996, Nature Struct. Biol. 3:206).

9. Use according to one of claims 1 to 8, in which the target protein is chosen from membrane-bound receptors coupled to the G protein.

10. Process for detecting and quantifying non-covalent interactions between the target protein, in particular a receptor, and one of its ligands, characterized in that:

– cells or cell fragments are prepared containing a DNA sequence comprising the gene coding for a fluorescent protein fused with the gene for the target protein, the fusion between the gene for the fluorescent protein and the gene for the above-mentioned target protein being such that the properties of the target protein, in particular of the receptor, are not modified by the presence of the fluorescent protein, namely:

* the interaction between the target protein, in particular the receptor, and the ligand is not modified,

* the response transduction function is not modified, the fluorescent protein being chosen from the fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, this protein being chosen in particular from:

– green fluorescent protein (GFP), or

– variants derived from GFP by addition, deletion or substitution of one or more amino acids, with the proviso that these variants conserve the fluorescence property,

– or fragments of GFP, or fragments of the above-mentioned variants, with the proviso that these fragments conserve the fluorescence property,

– the above-mentioned cells or the above-mentioned cell fragments are placed in contact with a ligand for the above-mentioned target protein, in particular for the above-mentioned receptor, labeled with a label consisting:

– either of a molecule capable of absorbing the light emitted by the fluorescent protein,

– or of a fluorescent substance,

and either the fluorescent protein being the fluorescence energy donor and the label being the fluorescence energy acceptor, or the fluorescent protein being the fluorescence energy acceptor and the label being a fluorescent substance which is a fluorescence energy donor, and

– irradiation is carried out at a wavelength which makes it possible either to excite the fluorescent protein or to excite the fluorescent substance,

– it being possible for the above-mentioned steps of placing in contact and irradiation to be carried out either simultaneously or one after the other, or

– the above-mentioned cells or the above-mentioned cell fragments are placed in contact with a ligand for the above-mentioned protein, in particular for the above-mentioned receptor, labeled with a label, the cells or the ligand having been irradiated before being placed in contact,

– either a reduction in the amplitude of the donor's emission and/or emission signal characteristic of the acceptor's emission is detected.

11. Process for detecting and quantifying non-covalent interactions between a target protein, in particular a receptor, and one of its ligands, characterized in that:

– a fluorescent protein fused with a target protein, the protein-ligand interaction of which it is desired to determine, is prepared, the fusion between the fluorescent protein and the above-mentioned target protein being such that the properties of the protein, in particular of the receptor, are not modified by the presence of the fluorescent protein, namely:

* the interaction between the target protein, in particular the receptor, and the ligand is not modified,

* the response transduction function is not modified,

the fluorescent protein being chosen from the fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, this protein being chosen in particular from:

– green fluorescent protein (GFP), or

– variants derived from GFP by addition, deletion or substitution of one or more amino acids, with the proviso that these variants conserve the fluorescence property,

– or fragments of GFP, or fragments of the above-mentioned variants, with the proviso that these fragments conserve the fluorescence property,

– the above-mentioned fluorescent protein fused with the target protein is placed in contact with a ligand for the above-mentioned protein, in particular for the above-mentioned receptor, this ligand being labeled with a label consisting:

– either of a molecule which is capable of absorbing the light emitted by the fluorescent protein,

– or of a fluorescent substance,

and either the fluorescent protein being a fluorescence energy donor and the label being a fluorescence energy acceptor, or the fluorescent protein being a fluorescence energy acceptor and the label being a fluorescent substance which is a fluorescence energy donor, and

– irradiation is carried out at a wavelength which makes it possible either to excite the fluorescent protein or to excite the fluorescent substance,

– it being possible for the above-mentioned steps of placing in contact and irradiation to be carried out either simultaneously or one after the other, or

– the above-mentioned fluorescent protein fused with the target protein is placed in contact with a ligand for the above-mentioned protein, in particular for the above-mentioned receptor, this ligand being labeled with a label consisting:

– either of a molecule which is capable of absorbing the light emitted by the fluorescent protein,

– or of a fluorescent substance,

the fluorescent protein fused with the target protein or the ligand having been irradiated before being placed in contact,

– either a reduction in the amplitude of the donor's emission and/or an emission signal characteristic of the acceptor's emission is detected.

12. Process according to claim 11, in which the fluorescent protein is EGFP and in which:

– either the EGFP is a fluorescence energy donor and the label is a fluorescence energy acceptor and is chosen from substances whose excitation spectrum overlaps the emission spectrum of EGFP, and in particular, when the label is a fluorescent substance, it is chosen from: 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (Bodipy), eosin, erythrosin, tetramethylrhodamine, sulphorhodamine 101 sold by Molecular Probe under the name Texas Red, and derivatives thereof which, on the one hand, allow grafting, and, on the other hand, have an excitation spectrum which overlaps the emission spectrum of EGFP,

and, when the label is not a fluorescent substance, it is chosen from the Acid Violet group [Acid Violet 5, CAS 10130-48-0 ; Acid Violet 7, CAS 4321-69-1 ; Acid Violet 17, CAS 4129-84-4], the Acid Red group [Acid Red 1, CAS 3734-67-6 ; Acid Red 8, CAS 4787-93-3 ; Acid Red 37, CAS 6360-07-2 ; Acid Red 40, CAS 12167-45-2 ; Acid Red 106, CAS 6844-74-2 ; Acid Red 114, CAS 6459-94-5], alizarins, aluminon, azocarmine B [CAS 25360-72-9], basic fuschin [Basic Red 9, CAS 569-61-9], Bordeaux R [Acid Red 17, CAS 5858-33-3] and Carmine [CAS 1390-65-4],

– or the EGFP is a fluorescence energy acceptor and the fluorescent substance is a fluorescence energy donor and is chosen from substances whose emission spectrum overlaps the excitation spectrum of EGFP, and in particular from: coumarins, fluorescamine, 6-(N-methylanilino)naphthalene , (mansyl) and derivatives thereof which, on the one hand, allow grafting, and, on the other hand, have an excitation spectrum which overlaps the emission spectrum of EGFP.

13. Process according to one of claims 10 to 12, in which the protein whose protein-ligand interaction it is desired to determine is chosen from:

– membrane-bound proteins coupled to the G protein, in particular in Supplement Trends in Pharmacological Sciences, 1997 (*Receptor and ion Channel Nomenclature*),

– growth factor receptors, in particular those which are structurally linked to the insulin receptor (Yarden, Y. and Ullrich, A. 1988, Biochemistry 27:3113-3119) or to the γ interferon receptor (Brisco, J. *et al.* 1996, Phylos. Trans. R. Soc. Lond. B. Biol. Sci. 351:167-171 ; Ihle, J.N. 1995, Nature 377:591-594),

– ion channel-receptors, in particular in Supplement Trends in Pharmacological Sciences, 1997 (*Receptor and ion Channel Nomenclature*),

– intracellular nuclear receptors, in particular those which are structurally linked to the steroid receptor (Mangelsdorf *et al.* 1995, Cell, 83:835-839; Wurtz, J.L. *et al.* 1996, Nature Struct. Biol. 3:206).

14. Process according to one of Claims 10 to 13, in which the fluorescent protein is EGFP and the labeled substance is Bodipy and in which either the reduction in the emission amplitude of EGFP or the emission signal of Bodipy resulting from the energy transfer is detected, the irradiation wavelength corresponding to the excitation wavelength of EGFP.

15. Process according to one of Claims 10 to 14, in which the fluorescent protein is EGFP and the labeled substance is a coumarin, and in which either the diminution of amplitude of coumarin or the emission signal of EGFP resulting from the energy transfer is detected, the irradiation wavelength corresponding to the excitation wavelength of coumarin.

16. Process according to one of Claims 10 to 15, in which the fluorescent protein is fused on the N-terminal side and the target protein, in particular the receptor, is fused on the C-terminal side.

17. Process according to one of claims 10 to 16, in which the fluorescent protein is fused on the C-terminal side and the target protein, in particular the receptor, is fused on the N-terminal side.

18. Process according to one of claims 10 to 17, in which the fluorescent protein is inserted into the target protein in a place not corresponding to a target protein-ligand binding sites, in particular in the case of receptors coupled to the G protein, this insertion taking place in the first or the third intracellular loop of the receptor, with the proviso that the insertion does not destroy either the properties of the receptor or the fluorescence of the fluorescent protein.

19. Process according to one of claims 10 to 18, in which the cells are mammalian cells, in particular HEK 293 cells which are adherent or in suspension, CHO cells, COS cells, lymphocytic lines, fibroblasts, etc., or yeast cells, in particular *pichia* such as *pichia pastoris*, *saccharomyces* such as *saccharomyces cerevisia, saccharomyces kluyveri, Hansenula* such as *Hansenula polymorpha*, or insect cells infected with a virus such as *baculovirus*, in particular TNI or sf9 cells, or fungi, in particular strains of *Aspergillus (A. oryzae, A. nidulans, A. niger*), *Neurospora, Fusarium* or *Trichoderma.*

20. Process according to any one of claims 10 to 19, in which a signal can be detected, in a conventional fluorimetry device or in a rapid-mixing device equipped with a system for detecting fluorescence, after mixing the donor and the acceptor, and can be abolished by the addition of a non-fluorescent substance of the same pharmacological specificity, and in particular in which the signal/noise ratio is a greater than about 2.

21. Cells or cell fragments containing a DNA sequence comprising the gene coding for a fluorescent protein fused with the gene for a target protein, the fluorescent protein being chosen from the fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, the fusion between the gene for the fluorescent protein and the gene for the above-mentioned target protein being such that

* the properties of the target protein are not modified by the presence of the fluorescent protein, that is to say

* the interaction between the target protein and the ligand is not modified,

* the response transduction function is not modified,

with the proviso that:

* when the target protein is the rat glucocorticoid receptor fused at the N-terminal with, successively, a purification sequence comprising 6 histidines, a haemaglutinin epitope and a fluorescent protein and is expressed in the cell line 1471.1, the fluorescent protein is other than GFP (768 base pairs of the plasmid TU65 with the mutation S65T),

* when the target protein is the human glucocorticoid receptor truncated of its first 131 amino acids, fused at the C-terminal of a fluorescent protein in the sites Sal I and BamHI and is expressed in the cells Cos-1, the said fluorescent protein is other than that GFP as described in the article by Inouye S. and Tsuji, F. I., 1994, Febs Letters, 341:277-280,

* when the target protein is the rat NMDA R1 sub-unit expressed in HEK 293 cells fused at the C-terminal with a fluorescent protein, the fluorescent protein is other than that consisting of the amino acids 2-238 of wild-type GFP,

* when the target protein is a receptor or a fragment of a receptor for intracellular second messengers, the fluorescent protein is other than that GFP and its derivatives.

22. Kit or equipment for detecting and quantifying non-covalent interactions between a target protein labeled with a fluorescent protein and one of its ligands labeled with a label consisting:

– either of a molecule which is capable of absorbing the light emitted by the fluorescent protein,

– or of a fluorescent substance,

this fluorescent protein being chosen from the fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, this protein being chosen in particular from:

– green fluorescent protein (GFP), or

– variants derived from GFP by addition, deletion or substitution of one or more amino acids, with the proviso that these variants conserve the fluorescence property,

– or fragments of GFP, or fragments of the above-mentioned variants, with the proviso that these fragments conserve the fluorescence property and its ligand labeled with a fluorescent substance, the said kit comprising:

– the target protein fused with a fluorescent protein or a stable cell line which is capable of expressing the protein fused with a fluorescent protein or a plasmid containing the nucleic acid sequence coding for the said targets protein fused with a fluorescent protein as defined above,

– the ligand labeled with the above-mentioned label,

– the buffers and media required for the energy transfer between the above-mentioned protein and the above-mentioned ligand.

23. Kit or equipment for detecting and quantifying non-covalent interactions between a target protein labeled with a fluorescent protein (No 1) and one of its ligands labeled with a fluorescent substance corresponding to a fluorescent protein (No 2), the fluorescent protein (No 1) being chosen from the fluorescent protein YP or EGFP and the ligand being labeled with a fluorescent protein (No 2) BFP, or the fluorescent protein (No 1) being BFP and the ligand being labeled with the fluorescent protein (No 2) YP or EGFP, the said kit comprising:

– either a plasmid containing a nucleic acid sequence coding for the target protein fused with a fluorescent protein (No 1), and

* a plasmid containing a nucleic acid sequence coding for the ligand fused with a fluorescent protein (No 2), or

* a ligand fused with a fluorescent protein (No 2), obtained via a recombinant route and purified,

– or a stable cell line which is capable of expressing the target protein fused with a fluorescent protein (No 1), and

* a stable cell line which is capable of expressing the ligand fused with a fluorescent protein (No 2) or

* a ligand fused with a fluorescent protein (No 2), obtained via a recombinant route and purified,

– the buffers and media required for the energy transfer between the above-mentioned protein and the above-mentioned ligand.